

Applicant : Daniel Pinto  
Serial No. : 09/877,935  
Filed : June 8, 2001  
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Attorney's Docket No.: 13294-002001

### REMARKS

Applicants submit a paper copy of the Sequence Listing as required under 37 CFR §1.823(a) and a verified statement under 37 CFR §1.821(f). In addition, Applicants have submitted herewith a Sequence Listing in computer readable form as required by 37 CFR §1.824. The amendments merely insert the paper copy of the Sequence Listing into the specification and add appropriate sequence identifiers to the specification. Applicants also have replaced the drawings in the specification with the enclosed drawings. Applicants submit that the enclosed drawings comply with 37 CFR §1.84. No new matter has been added. Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date:

February 19, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph on page 9, line 22 through page 10, line 7, has been amended as follows:

**Determination of the transcription start site of the mouse villin gene by primer extension.**

A, primer extension analysis was performed with mouse intestinal total RNA (30 µg) and with either the end-labeled villin oligonucleotide (generating a 105-nucleotides extension product) or the end-labeled mouse intestinal fatty acid binding protein gene (Fabpi) oligonucleotide used as a positive control (generating a 81-nucleotides extension product). The size of the fragments obtained by primer extension is shown at the left. The unrelated sequence ladder that was run in the same gel is used as a size marker. B, nucleotide sequence between the transcription start site (the bold adenosine designated as +1) and the initiation codon (the bold underlined ATG codon) of the mouse villin cDNA (SEQ ID NOs:11 and 12). Each of the splice junctions present in the intron 1 (indicated below) conforms to the consensus splice donor (the italicized GT nucleotides) and acceptor (the italicized AG nucleotides) patterns, described by Breathnach and Chambon (30). C, schematic representation of the organization of the 5'-flanking region of the murine villin gene. The open box represents the untranslated exon and the shadowed box represents the first coding exon. The size of the exon and the intron is indicated.

The paragraph on page 12, lines 2-7, has been amended as follows:

Sequence of the genomic DNA of the murine villin gene (SEQ ID NO:1) comprising cis-acting elements capable to promote the transcription of the murine villin gene in intestinal mucosa and kidney proximal tubules. The sequence comprises the transcription initiation site at position 3442 followed by the sequence of exon 1 containing 46 pb, the translation initiation codon at position 8993, the sequence of intron 1 extending from nucleotide 3488 to nucleotide 8981.

The paragraph on page 13, line 26 through page 14, line 13, has been amended as follows:

Total RNA was isolated from mouse intestine with RNA NOW reagent (Biogentex) under the conditions suggested by the supplier. For primer extension assay, 2 ng of <sup>32</sup>P-labeled oligonucleotide probe (5'-GAGTGGTGATGTTGAGAGAGCCT-3'; SEQ ID NO:2) complementary to nucleotides +81 to +103 of the murine villin cDNA (GenBank Accession No.

M98454) was hybridized with 30 µg of total RNA at 60°C (0.25 M KCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA) for 90 min. Transcription with 5 U/µl of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) was carried out at 37°C for 90 min in a 300 µl of a solution containing 75 mM KCl, 3 mM MgCl<sub>2</sub>, 50 mM Tris-HCl (pH 8.3), 10 mM dithiothreitol, 0.75 mM deoxynucleoside triphosphates, 75 µg/ml actinomycin D and 0.3 U/µl RNasin. The primer extension products were separated by electrophoresis in denaturing 8% polyacrylamide gels. The full-length extension product (105 nucleotides) was obtained by comparison with the length of the comigrating sequencing reaction products. A primer extension control experiment was performed on the same total RNA preparation, using a <sup>32</sup>P-labeled oligonucleotide probe (5'-CATAGTTCTCGTTCCGGT-3'; SEQ ID NO:3) complementary to nucleotides +63 to +80 of the mouse intestinal fatty acid binding protein (I-FABP) cDNA and generating a 81-nucleotide extension product (27).

The paragraph on page 15, line 3 through page 16, line 2, has been amended as follows: All constructs described were subcloned into the pBluescript II KS vector (Stratagene) with fragments isolated from a λDASHII phage containing a 16.3 kb region (9 kb upstream and 7.3 kb downstream from the translation initiation codon) of the mouse villin gene (29). The pD1 construct (as described in the Fig. 3B) was prepared by ligating a *Bam*HI fragment of 5.1 kb (1.8 kb upstream from the ATG translation initiation codon of the mouse villin gene, subcloned 5' to the nuclear localization signal-β-galactosidase gene-SV40 polyadenylation site, using a polymerase chain reaction (PCR) strategy) at the *Bam*HI site in a plasmid containing the 3.7 kb region of the mouse villin gene (immediately 5' to the 1.8 kb region described above). The pA1 and pA2 (containing an internal 1 kb deletion) constructs have resulted from several steps based on the *Bst*EII sites present in the 3.7 kb region described above and in a plasmid containing the 3.5 kb region of the mouse villin gene (immediately 5' to the 3.7 kb region). The pC1 and pC2 constructs were derived from the pA1 and pA2 plasmids cut with *Apa*I and re-ligated, respectively. To generate the pB1 construct, a *Bgl*II fragment (480 bp) from the 3.5 kb region described above was excised and cloned into the *Kpn*I site of the pC1 plasmid. The pA3, pB3 and pC3 constructs correspond to the pA1, pB1 and pC1 deleted from the intron 1 (Fig. 3B). The sequence between the transcription initiation start site and the translation initiation codon, excluding the intron 1, was deduced from that of the murine villin cDNA (GenBank Accession

No. M98454) and was introduced into the *Bgl*II-*Nco*I sites of the pC1 construct by using a dimerized oligodimer made of a coding-strand oligonucleotide (5'-GATCTCCCAGGT GGTGGCTGCCTCTTCCAGACAGGCT CGTCCAC-3'; SEQ ID NO:4) and a non coding-strand oligonucleotide (5'-CATGGTGGACGAGCCTGTCTGGAAGAGGCAGCCACC ACCTGGGA-3'; SEQ ID NO:5), resulting in the pB3 construct. The pA3 and the pC3 constructs were derived from the pB3 plasmid by ligating an *Apal* fragment (3.1 kb) and a *Bgl*II fragment (480 bp) from the 3.5 kb region described above, at the *Apal* site in the pB3 plasmid respectively. Subcloning steps were confirmed by DNA sequencing.

The paragraph on page 16, line 19 through page 17, line 8, has been amended as follows: Total RNA was isolated from mouse tissues described above, with SV Total RNA Isolation System (Promega) under the conditions suggested by the supplier. 20 ng of pd(N)<sub>6</sub> random primer (Pharmacia) were hybridized with 2 µg of total RNA at 70°C for 10 min in distilled water. Reverse transcription with 200 U of Moloney murine leukemia virus reverse transcriptase (SuperScript II, Life Technologies, Inc.) was carried out at 37°C for 90 min in a 20 µl solution of 1X First Strand Buffer (Life Technologies, Inc), 10 mM dithiothreitol, 0.5 mM deoxynucleoside triphosphates and 0.4 U/µl RNasin. 2 µl of the resulting cDNAs, were amplified by PCR reaction in 50 µl for 40 cycles. Each cycle consisted of 60 sec at 94°C, 60 sec at 51°C (for transgene and villin) and 57°C (for TFIID), and 30 sec at 72°C. For the transgene primers, 5'-CAACTTCCTAAGATCTCC-3' (SEQ ID NO:6) coding strand and 5'-ATTCAGGCTGCG CAACTGTT-3' (SEQ ID NO:7) non-coding strand were used, generating a 250 bp product. For villin amplification 5'-CAACTTCCTAAGATCTCC-3' (SEQ ID NO:6) coding strand primer and 5'-GCAACAGTCGCTGGACATCACAGG-3' (SEQ ID NO:8) non-coding strand primers were used, generating a 473 bp product; for TFIID amplification 5'-CCACGGACAACTG CGTTGAT-3' (SEQ ID NO:9) coding strand primer and 5'-GGCTCATAGCTACTGAACTG-3' (SEQ ID NO:10) non-coding strand primer were used, generating a 220 bp product. In all cases, one-fifth of the PCR product was run on an ethidium bromide containing agarose gel.

The enclosed paper copy of the Sequence Listing has been added to the specification after the drawings.

#### In the Drawings

The drawings in the application have been replaced with the enclosed drawings.